In support of the objection, the Office Action refers to the unpredictable and complex nature of the subject matter, stating that "the claimed invention is unlikely to be accepted in the absence of clear and convincing data." The Office Action then cites numerous sources for the proposition that genetic immunization is unpredictable. Applicants respectfully submit that the lack of results presented by others should have no bearing on the patentability of the claimed invention. As discussed below, the examples clearly support every step recited in the claims and the results achieved by these steps. In any event, many of the references link the unpredictability of gene therapy in general with the inability to achieve extended expression of a gene for long periods of time. The genetic immunization methods claimed by Applicants, however, require that the DNA fragment have only limited expression, which requires a much shorter time period for expression than the time period needed for the gene therapy discussed in the references. Applicants respectfully submit, therefore, that these references are inapposite. Furthermore, none of these references state or even suggest that the type of genetic immunization claimed in the present invention is unpredictable.

For example, Schofield and Caskey are clearly discussing long term gene expression, and state that there is a wide variation in gene expression when using particle bombardment. The reference also indicates, however, "that particle bombardment is an effective means of gene transfer both *in vitro* and *in vivo*" and that particle bombardment may be useful in vaccination or immunization protocols. (See pages 57 and 59). Other references also support the use of particle bombardment in genetic immunization. (See, for example, Eisenbraun.) It is the use of particle bombardment for genetic immunization, and not long term expression, that is contemplated by Applicants. Moreover, although the reference is critical of particle bombardment that requires a surgical procedure, Applicants discuss cutaneous bombardment, which does not involve a surgical procedure.

For similar reasons, Applicants respectfully submit that the Marshall article does not lend support to the 112 rejection. Again, this article is directed towards gene therapy requiring long term gene expression, and not to gene immunization requiring short term gene expression. Furthermore, the fact that others have had ambiguous results in showing therapeutic benefits, does not mean that Applicants have failed to provide adequate evidence of a therapeutic benefit or

that they have failed to provide an enabling disclosure to achieve that benefit. The same can be said about the Coghlan and Brown references. The comments made in these articles simply are not relevant to the genetic immunization disclosed and claimed by Applicants.

The Office Action next cites to the Bignon reference stating that in all of the gene immunotherapy trials reported therein, therapeutic efficiency was lacking. Again, Applicants respectfully submit that this is not a basis for the rejection of the present application. As is discussed below, Applicants have demonstrated a therapeutic benefit according to the methods of the present invention. In addition, the statement in Bignon that "the lack of immunoreactivity in spontaneous cancers would be due, more to a lack of knowledge of the way to become immunized against these antigens" actually supports Applicants disclosure; the reference clearly identifies a problem in the art that the Applicants have addressed, namely a way to effect immunization. It is because of problems such as those reported in Bignon, that the Applicants devised the novel method of genetic immunization reported in the present invention.

The Office Action concedes that the working examples "illustrate genetic immunization by biolistic administration or subcutaneous administration of the particulate polynucleotide" but then states that the examples are nonetheless "clearly deficient" in supporting many aspects of the claimed invention. More specifically, the Office Action alleges that the working examples only support a use of particulate polynucleotide comprised of the pAc-Neo-OVA, while the claims read on employment of any type of particulate polynucleotide expressing an antigenic protein or fragment thereof. The Office Action further notes that "melanoma B17 [sic, B16] and OVA peptide . . . are well known in the art." It is precisely for this reason that the Applicants chose to use the OVA antigen in their studies. As discussed in the specification at page 23, lines 3-7, the B16 melanoma is extensively studied, ovalbumin has a well defined structure and the intracellular processing and presentation of OVA in the C57 BL/6 mouse is known. By employing an antigen whose behavior was well known, the full effect of the methods of the present invention could be monitored and demonstrated as effective. Furthermore, the OVA antigen is representative of all of the other antigens disclosed in the application.

The Office Action is further critical of the working examples in that they show support only for a mouse model. Cournoyer and Caskey are cited to support the contention that results in animals are variable. It is well established that animal models can be used to demonstrate efficacy for other mammalian hosts, including humans. The mouse model is well-defined and is particularly appropriate here. Because the biology of the processing and presentation of the OVA antigen used in this model is the same as that of many naturally occurring tumor antigens, the results obtained here are applicable to a wide variety of tumors. Furthermore, the mouse model is widely used and reported in numerous other sources as well. (See, for example, Eisenbraun, Cournoyer and Caskey, and Bignon and references cited therein.)

The Office Action also states that the application is deficient in failing to specify the dosage involved for a therapeutic effect. The Examples discuss the amount of polynucleotide injected in the mice; example section 7 clearly states that the mice were injected with 2.64 micrograms of OVA encoding DNA. In addition, as will be appreciated by one skilled in the art, the amount of any given therapeutic agent will depend on the person being treated and the illness being treated. It is within ordinary experimentation, therefore, to extrapolate results provided in an animal model to a human model.

Applicants respectfully submit that the specification, including the working examples, clearly enable one skilled in the art to practice the claimed invention.

Example Section 6 demonstrates expression of a transfected antigen according to the methods of the present invention. This example used the OVA/B16 murine model, which was also used in Example Sections 7 and 8.

Example Section 7 demonstrated the efficacy of the present invention using injection by a biolistic device. More specifically, Example 7 teaches generating a DNA fragment which expresses an antigenic protein or fragment thereof and distributing said DNA fragment on a particle surface, thus creating a particulate polynucleotide. For example, DNA coated gold particles can be prepared by combining gold beads with spermidine, followed by the desired plasmid DNA. (See specification page 25, lines 10-16.) Furthermore, the Example clearly enables one skilled in the art to prepare and administer these golds beads;

inoculating a mammalian host with said particulate polynucleotide can be accomplished by use of a biolistic device, by directed inoculation, or by effecting *in vitro* transfection of target cells. (See specification, page 25, line 16 through page 26, line 24.) Inoculation results in delivery of the particulate polynucleotide to the cytoplasm of a target cell, and the antigenic protein is presented to the membrane surface of said target cell through the MCH class 1 pathway, to result in genetic immunization of the host. That this result is achieved by these methods is further demonstrated by the examples. Following immunization, the mice were challenged with tumors. The efficacy of biolistic immunization was evaluated with immunization naive C57 BL/6 mice injected with about 2.64 micrograms of OVA encoding DNA, and identically boosted 7 days after initial immunization. Seven days later, the mice were injected at a distant site with the MO4 melanoma. OVA immunized mice were protected from lethal tumor challenge, while tumors in control mice grew progressively. (See page 27, line 19 through page 28, line 4.) Thus, the example demonstrates delivery of the particulate polynucleotide to the cytoplasm of a target cell of a host so that the expressed antigenic protein was presented on the membrane surface of the target cell and a prophylactic genetic immunization effect was realized.

Example Section 8 demonstrates the ability of the methods of the present invention to specifically target phagocytic APCs in the lymphoid tissue, or APCs capable of trafficking to the lymphoid tissue. This example utilized subcutaneous injection of particulate polynucleotides *in vivo* as discussed in Example Section 7. As demonstrated by Example Section 8, subcutaneous injection and biolistic administration yielded essentially equal protection from tumor challenge. Because the target cells were not directly injected, or "bombarded", with the particles, as in Example Section 7, this example further shows that the polynucleotides were actually being taken up by host cells, namely APCs, capable of phagocytosis/endocytosis. The demonstrated tumor protection of these cells shows that the polynucleotides were not only taken up, but that the DNA on the polynucleotides was expressed by the cells.

In addition, the attached Declaration of co-inventor Louis D. Falo explains how he repeated the methodology set forth in the examples, and obtained the same or similar results, confirming that the specification is enabling, that the

claimed results can be achieved by employing the methods as disclosed in the present invention, and that these methods and results are reproducible.

The methods outlined in the examples were essentially repeated, as detailed in the Declaration and Exhibit II attached thereto. By following these methods, genetic immunization with DNA encoding an antigenic protein or fragment thereof results in potent, antigen-specific, cytotoxic T lymphocyte-mediated protective tumor immunity. Moreover, the methods of the present invention have been proven to result in expression of a protein in lymph nodes following biolistic injection of particulate polynucleotides in cutaneous dendritic cells. Thus the specification enables one skilled in the art to practice the invention without undue experimentation.

For all of the above reasons, Applicants respectfully submit that the specification is enabling for the invention as claimed.

Claims 1 through 67 were also rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification. For all of the reasons given above, Applicants respectfully submit that this rejection has been overcome.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claim 59 was rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. More specifically, the terms "biologically significant form" and "biologically significant levels" were deemed vague and indefinite. The claim has been amended to delete the phrase "in a biologically significant form" and to change the phrase "biologically significant levels" to "biologically effective levels". "Biologically effective levels" are clearly referred to in the specification as levels "such that antigenic peptide fragments are processed and presented through the endogenesis MHC class one pathway, and displayed on the membrane surface of the target cells." (See, for example, specification page 18, lines 13-16 and page 19, lines 14-17.) Applicants respectfully submit, therefore, that the rejection has been overcome.

Rejections Under 35 U.S.C. § 103

Claims 1-67 were rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Nabel et al. taken with Eisenbraun et al. and further in view of Robinson et al. This rejection is respectfully traversed.

Nabel is directed to an immunotherapy in which genes encoding an MHC glycoprotein are introduced *into tumor cells*. Nabel reports that this induces cytolytic T-cells and produces partial tumor regression. Thus, Nabel is claiming a therapeutic benefit by transfecting the tumor itself. In contrast, the present invention achieves a therapeutic benefit by delivery of a particulate nucleotide containing a DNA encoding an antigenic protein or fragment thereof to the cytoplasm of a target cell, preferably an APC. Following transfection of the target cells, the immunizing protein is produced intracellularly and hence has access to the MHC class one restricted presentation pathway. Naturally processed epitopes result, and the transfected cells produce the immunizing protein. Thus the present invention functions in a much different manner than that reported by Nabel.

As noted in the Office Action, Nabel uses DNA/liposome transfection, rather than particle bombardment. To overcome this deficiency in the primary reference, the Office Action further cites Eisenbraun. Eisenbraun is generally directed to intercellular delivery of a human growth hormone expression construct by using particle bombardment-mediated gene transfer. Eisenbraun is a proponent of the use of particle bombardment for gene delivery. Eisenbraun, however, does not remotely teach or suggest the use of particle bombardment in the manner taught by Applicants in, for example, Claims 15 through 28. Specifically, Eisenbraun is silent as to generation of a DNA fragment which expresses an antigenic protein or fragment thereof, creating a particulate polynucleotide containing said DNA fragment, and delivering said particulate polynucleotide to the cytoplasm of a target cell within said mammalian host, such that said expressed antigenic protein or fragment thereof is presented to the membrane surface of said target cell through the MHC class one pathway. Furthermore, Applicants are not claiming as novel the use of particle bombardment itself, but rather the use of particle bombardment in the manner described above to achieve the results as claimed. Eisenbraun is silent as to this methodology and these results.

Moreover, Eisenbraun and Nabel taken in combination do not teach or suggest delivery of a particulate polynucleotide containing a DNA fragment expressing an antigenic protein or fragment thereof to the cytoplasm of a target cell such that the antigenic protein or fragment thereof is expressed and presented to the membrane surface of the target cell through the MHC class one pathway. As stated above, Nabel teaches introduction of genes encoding a glycoprotein into the tumor itself. Even if this introduction was accomplished through particle bombardment, the present invention would still not be taught by the combination of references.

The Office Action also cites to Robinson et al., who use direct DNA inoculation of an H7 haemagglutinin type 7 (H7) gene for immunization against an influenza virus. As with the particle bombardment methodology, Applicants are not claiming the novelty of direct inoculation as a means of distributing DNA fragments. It is the use of inoculation to result in the expression of antigenic proteins or fragments thereof and presentation of these proteins to the membrane surface of a target cell through the MHC class one pathway in antigen presenting cells to achieve the claimed results that are both novel and nonobvious over the art. Robinson simply does not teach this methodology or its result. In addition, Robinson is silent as to inoculation using particulate polynucleotides.

Moreover, Robinson and Nabel taken in combination do not teach or suggest delivery of a particulate polynucleotide containing a DNA fragment expressing an antigenic protein or fragment thereof to the cytoplasm of a target cell such that the antigenic protein or fragment thereof is expressed and presented to the membrane surface of the target cell through the MHC class one pathway. As stated above, Nabel teaches introduction of genes encoding a glycoprotein into the tumor itself. Even if this introduction was accomplished through direct inoculation, the present invention would still not be taught by the combination of references.

Nabel teaches a decidedly different methodology in which the tumors themselves are transfected with genes encoding a glycoprotein. As discussed above, this is significantly different from Applicants' claimed invention. The citation of Eisenbraun and Robinson for the proposition that particle bombardment and direct inoculation are known does not overcome the shortcomings of the Nabel reference. Accordingly, Applicants respectfully submit that the present invention is not obvious over the three references taken alone or in any combination. Combining the three

references in the manner suggested in the Office Action simply would not yield the present invention as claimed by Applicants.

Furthermore, there is no suggestion in any of these references, when considered singly or in combination, of providing the methods of therapeutic or prophylactic genetic immunization as claimed by Applicants. Effecting such a combination of components and features as found in Claims 1-67 would require significant destruction of the teachings of Nabel, Eisenbraun and Robinson Furthermore, for a combination of references to be properly applied, the combination must suggest an improvement along the lines of the invention to one skilled in the art. (See, for example, In re Sernaker, 217 U.S.P.Q. 1 (Fed. Cir. 1983)). Here, Applicants cannot discern any suggestion whatsoever that the combination proposed in the Office Action would lead to the improved methodologies of the present invention.

Claim 1 is directed to an *in vivo* method of therapeutic or prophylactic genetic immunization of a mammalian host comprising generating a DNA fragment which expresses an antigenic protein or fragment thereof, distributing the DNA fragment on a particle surface resulting in a particulate polynucleotide; inoculating said host with said particulate polynucleotide; and delivering said polynucleotide to the cytoplasm of a target cell within the host, such that the expressed antigenic protein or fragment thereof is presented to the membrane surface of the target cell through the MHC class one pathway. As discussed above, none of the art, taken alone or in combination, teaches or suggests the combination of steps in the method of Claim 1.

Claim 2 depends from Claim 1 and further defines said mammalian host as a human. Claim 3 depends from Claim 2 and further defines said DNA fragment as a tumor rejection antigen, a viral antigen or an antigenic protein fragment thereof. Again, none of the references teach or suggest the combination embodied in these claims.

Claim 4 depends from Claim 3 and further defines said target cell as an antigen presenting cell. As discussed above with regard to the Nabel reference, the art is directed to targeting a tumor cell and not an antigen presenting cell. Applicants respectfully submit, therefore, that the combination embodied in Claim 4 is not taught or suggested by the art. Nor is the combination of elements presented

in Claim 5 suggested in the art. Claim 5 depends from Claim 4 and defines said antigen presenting cell as residing within or migrating to the lymphoid tissue of the human host.

Claim 6 depends from Claim 5 and further defines the tumor rejection antigen as being selected from the group consisting of MAGE-1 and MAGE-3. Claims 7-11 also depend from Claim 5 and further define the tumor rejection antigen as Melan-A, gp100, p53, CEA, and HER2/neu, respectively. Claims 12-14 also depend from Claim 5 and further define the viral antigen as HIV gp120 HIV gp160, influenza virus nucleoprotein, or Hepatitis B surface antigen, respectively. None of the art teaches or remotely suggests the use of these tumor rejection antigens or viral antigens in the manner taught by these claims.

For all of the above reasons, Applicants respectfully submit that Claims 1-14 are allowable over the art.

Claim 15 is similar to Claim 1, but requires that the inoculation of the host be effected by using a biolistic device. Claims 16-28 all depend, directly or indirectly, from Claim 15 and are analogous to Claims 2-14. As stated above with regard to the Nabel and Eisenbraun references, the art does not teach or suggest the use of a biolistic device in the method disclosed in Claim 15. Nor do any of the references, taken alone or in combination, teach or suggest the combinations claimed in Claims 16-28. For all of these reasons, Applicants respectfully submit that Claims 15-28 are allowable over the art.

Claim 29 is similar to Claim 1 but requires that inoculating the host be accomplished by direct injection. Claims 30-43 depend, directly or indirectly, from Claim 29 and are analogous to Claims 2-14. As discussed above with regard to the Nabel and Robinson references, the art does not teach or suggest the use of direct injection in a method such as that claimed in Claim 29. Moreover, none of the references taken either alone or in combination teach or suggest the combination embodied in Claim 30-43. For all of these reasons, Applicants respectfully submit that Claims 29-43 are allowable over the art.

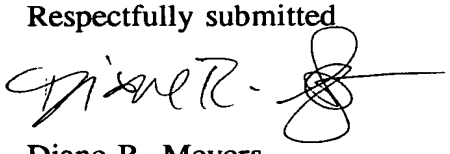
Moreover, none of the references appear to teach or suggest the *ex vivo* methods of therapeutic or prophylactic genetic immunization taught in Claims 44-67. Accordingly, Applicants respectfully submit that these claims are therefore allowable.

For all of the above reasons, Applicants submit that one skilled in the art would not have been motivated to combine the references in the manner suggested in the Office Action, and that all of the claims are therefore nonobvious over these references. Reconsideration of the rejection and allowance of Claim 1-67 is respectfully requested.

SUMMARY

In summary, it is respectfully submitted that Applicants' Claims 1-67 are allowable as they are all patentably distinct from the cited art. Furthermore, Applicants respectfully submit that the specification, as well as the claims, clearly enable one skilled in the art to practice the invention. Claim 59 has been amended to overcome the rejection under 35 U.S.C. § 112, second paragraph. Applicants therefore respectfully submit that the application is in proper form for issuance of a notice of allowance, and such action is respectfully requested at an early date.

Respectfully submitted



Diane R. Meyers
Registration No. 38,968
Attorney for Applicants

(412) 566-2036